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Prevalence of parasitemia and associated immunodeficiency among HIV-malaria co-infected adult patients with highly active antiretroviral therapy

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ABSTRACT

Objective: To investigate the malaria parasitemia, $CD4^+$ cell counts and some haematological indices among HIV-malaria co-infected adult patients with highly active antiretroviral therapy (HAART). **Methods:** A total of 342 adult HIV positive subjects were recruited at the consultant outpatient HIV/AIDS clinic, University of Benin Teaching Hospital, Benin City, Nigeria between June 2011 to November 2011. Blood samples were taken for malaria parasite count, $CD4^+$ cell count and other haematological counts. **Results:** Out of the 342 adult HIV positive subjects a total of 254 patients (74.3%) were found to have malaria parasitemia. The incidence of malaria parasitemia increased with advancing clinical stage of HIV infection and this was statistically significant (P=0.002). There was no statistical significance when gender was compared with the HIV-malaria status (P >0.05). Of the 254 co-infected patients, 134 (52.8%) had high parasitemia (>1.25×10^9/L). Sixty patients were found to be hyperparasitemic (>2.5 parasites/L). There was a significant association between $CD4^+$ cell count and having significant parasitemia (P < 0.000 1). About half (50.8%) of co-infected patients had $CD4^+$ cell count $\leqslant$ 200/ μ L, and majority (44.9%) of this population also had significant parasitemia. Anaemia and thrombocytopenia were not significantly associated with HIV-malaria co-infection (P > 0.05). **Conclusions:** The prevalence of parasitemia is high among the HIV/AIDS infected patients.

1. Introduction

Malaria and HIV/AIDS are two disease conditions that are of great public health concern in sub-Saharan Africa. The dual infection of HIV and malaria fuels the spread of both diseases in sub-Saharan Africa. Malaria is responsible for more than 1 million deaths worldwide every year with about 90% occurring in sub-Saharan Africa[1,2]. Also an estimated 28 million individuals are currently infected with HIV in sub-Saharan Africa with almost 3 million deaths annually[3]. Expectedly, the overlap in the distribution of these two disease conditions makes co-infection and interaction inevitable even as morbidity and mortality increases.

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In East and Southern Africa where HIV prevalence is near 30%, it is estimated that by the end of 2009, an estimated 3.3 million people would be living with HIV in Nigeria[4]. Nigeria is considered an area of stable *Plasmodium falciparum* transmission. Her adult prevalence of HIV has dropped from 5.8% in 2001 to 4.4% in 2005[5] and 21%-28% of people living with HIV/AIDS are co-infected with malaria[6,7].

Previous studies have shown evidence of detrimental relationship between the two disease conditions especially in areas where they are co-endemic[6-9]. On one hand, HIV infection has been reported to roughly double the risk of malaria parasitemia and increase the frequency and severity of clinical malaria[8,9]. On the other hand, clinical malaria has been reported to facilitate the HIV replication through incitement of cytokine production and immune-cell activation[10-12]. The increased rate of HIV replication

leads to a lasting elevation in viral load, which in turn hasten immune system decline and HIV disease progression to AIDS and death or increase the risk of its transmission[13-15].

The potential risks of adverse drug interactions have critical implications for effective management of co-infection. Such interactions may result in treatment failures, especially with sulfa-based drugs, or toxicity. While antiretroviral protease inhibitors have demonstrated some antimalarial effects, co-trimoxazole prophylaxis have been reported to prevent malaria episodes in HIV-infected patients[16]. Therefore identification of the best methods for reducing the incidence of malaria in adults with HIV infection and better understanding of the relationship between HIV disease and malaria could improve clinical and public health strategies. The aim of this paper is to study the prevalence of malaria parasitemia, $CD4^+$ cell count and some haematological indices among co-infected HIV/malaria individual on highly active antiretroviral therapy (HAART) in an endemic region for both disease.

2. Materials and methods

2.1. Participants and study design

The study group comprised 342 adult HIV subjects with HAART attending the consultant outpatient HIV/AIDS clinic in the University of Benin Teaching Hospital, Benin City, Nigeria between June to November 2011. The hospital has a 700 bed capacity and is a major referral and treatment centre for the South-South geopolitical zone of Nigeria where two major HIV/AIDS treatment units exist: the Federal Government Sponsored Project and President Emergency Plan for AIDS Relief, USA.

After informed consent was obtained, individuals attending the clinic had detailed clinical history and examination, and biodata documented. Each patient was assigned an HIV stage according to the proposed WHO hierarchical staging classification based on clinical and performance criteria. For the purpose of this study they were grouped into asymptomatic (stage 1 and 2) and symptomatic (stage 3 and 4) staging system of HIV/AIDS for Africa[17].

2.2. Laboratory methods

All participants had regular $CD4^+$ cell count and complete blood count measurements as part of routine workup to assess the disease severity. The haematological counts were determined by automation and $CD4^+$ cell counts were counted by flow cytometry. The HIV status of each participant was already determined by screening their plasma samples with two parallel rapid tests for detecting HIV Ⅰ and Ⅱ antibodies (Abbott determine 1 & 2 and Capillus HIV-1/HIV-2) following the manufacturers' instructions. Samples concordantly positive or negative on both assays were not tested further.

Thick and thin peripheral blood smears for malaria parasites were prepared from capillary blood harvested through finger prick on each subject, stained with 3% Giemsa stain at pH 7.2 for 30 minutes and was examined under ×100 oil-immersion fields. Initially, the thin blood film was screened by observation of 100 fields, each representing about 200 erythrocytes, and the result was then adjusted to 5 million erythrocytes per one microliter to obtain the parasite count. In case of negative thin film, the parasite: leukocyte ratio was measured on the observation of 1 000 leukocytes in the thick blood film, and the total white blood cell count of each participant was used in calculating the parasite count. Non-falciparum plasmodium species were excluded. A slide was positive for malaria with any level of parasitemia and significant parasite count at cut-off of 1.25×10^9 parasites/L[8]. Anemia was defined as Hb<10 g/dL and thrombocytopenia as platelet count less than 150×10^9/L. All the participants investigated were already on HAART therapy and appropriate antimalaria drug regimen was given when diagnosis of malaria parasitemia was made. None of the patient was on antimalaria prophylaxis.

The study was given ethical approval by the hospital. The first-line ART treatment regimen consisted of stavudine, lamivudine and either nevirapine or efavirenz. For participants who later developed clinical or laboratory evidence of ART treatment-failure or toxic effects, available second-line agents were didanosine, zidovudine, tenofovir and emtricitabine.

3. Results

A total of 342 adult HIV infected patients were recruited during the study period. This comprised of 90 males (26.3%) and 252 females (73.7%) with a male-to-female ratio of 1:2.8. The overall mean age of the patients was (40±10) years with a median of 39 years. The baseline demographic characteristics of the subjects are captured in Table 1. Majority of the patients (39.2%) were in the age bracket 31-40 years with a female preponderance. A total of 214 (62.6%) participants were asymptomatic, presenting in stage 1 and 2, while 128 (37.4%) symptomatic patients presented in advanced stage disease (3 and 4). Of the 342 participants, 134 (39.2%) had a $CD4^+$ cell count less than 200 cells/μL, 42 (12.3%) were in 201-350 cells/μL, 54 (15.8%) in 351-500

cells/ μ L, and 112 (32.7%) were >500 cells/ μ L.

Table 1
Baseline information on study population.

Age (years)	Male (%)	Female (%)	Total (%)
<20	2 (0.6)	4 (1.2)	6 (1.8)
21–30	6 (1.8)	43 (12.6)	49 (14.3)
31–40	28 (8.2)	106 (31.0)	134 (39.2)
41–50	36 (10.5)	53 (15.5)	89 (26.0)
51–60	14 (4.1)	45 (13.2)	59 (17.3)
>60	4 (1.2)	1 (0.3)	5 (1.5)

Table 2 revealed that 254 subjects (74.3%) were found to have malaria parasitemia. This gives a negative smear for *Plasmodium falciparum* in 25.7% of the study patients. A total of 214 (62.6) with HIV progression were symptomatic (stage 3–4), out of which 147 (43.0%) had parasitemia. The odds of having malaria parasitemia increased with advancing clinical stage of HIV infection and this was statistically significant (P=0.002, OR=2.32). The 254 co-infected HIV and malaria infections had a mean age of 39.8±9.7. There was no statistical significance when gender was compared with the malaria status (P >0.05).

There was a significant association between $CD4^+$ cell count ≤200 cells/ μ L and having significant parasitemia (P < 0.0001) as shown in Table 3. About half (50.8%) of co-infected patients had $CD4^+$ cell count ≤200 cells/ μ L with majority (44.9%) of this population having significant parasitemia. The odds of significant parasitemia increased with falling $CD4^+$ cell count. Individuals with $CD4^+$ cell count≤200 cells/ μ L had higher risk of malaria parasitemia compared to those with $CD4^+$ cell count >200 cells/ μ L.

The selected blood findings obtained from the co-infected patients are shown in table 4. The overall mean parasite count was $(2.37\pm0.20)\times10^9$/L with a median of 1.3×10^9/L. Of the 254 co-infected patients, 134 (52.8%) had high parasitemia (>1.25×10^9/L) with a mean parasite count of $(3.78\pm0.37)\times10^9$/L. Sixty patients were found to be hyperparasitemic (>2.5×10^9/L). The prevalence of anaemia (Hb≤10g/dL) in HIV and malaria co-infected patients was 33.1% with a mean value of (9.15±0.11) g/dL while 21.7% were thrombocytopenic (≤150×10^9/L) with a mean of (107.5±4.4)$\times10^9$/L and a range of (33–150)$\times10^9$/L.

4. Discussion

Mounting evidence has revealed pathological interactions between HIV and malaria in dually infected patients, but the implications of the interplay have remained unclear. Given the extensive overlap in the geographic distribution of malaria and HIV infections, even modest interactions between them would have enormous public health importance. Recent evidence clearly supports presence of the significant impact of each infection on the other and by extension on the individuals[18,19]. Majority of the HIV subjects were within the age bracket 31–40 years, representing the economic age bracket and this has serious socio-economic impact on the nation.

The $CD4^+$ T cells are crucial in immune responses against HIV virus and malaria parasite infection. *Plasmodium falciparum* has been shown to stimulate HIV replication through the production of cytokines (inteleukin 6 and tumor necrosis factor alpha) by activated lymphocytes[11,12]. Our study revealed that 39.2% of the subjects had low CD4 cell

Table 2
Clinical stage and gender prevalence of *Plasmodium* malaria infection in study population.

Characteristic		Malaria infected (%)	Malaria uninfected (%)	Total (%)
Clinical stage	Asymptomatic (1–2)	107 (31.3)	21 (6.1)	128 (37.4)
	Symptomatic (3–4)	147 (43.0)	67 (19.6)	214 (62.6)
Gender	Male	72 (21.1)	18 (5.3)	90 (26.3)
	Female	182 (53.2)	70 (20.5)	252 (73.7)

Table 3
Relationship between CD4 cell count and significant parasitemia among co-infected patients (n = 254).

$CD4^+$ cell count(/ μ L)	Significant parasitemia (≥1.25×10^9/L)	Non significant parasitemia (<1.25×10^9/L)	Total
≤200	114 (44.9%)	15 (5.9%)	29 (50.8%)
>200	20 (7.8%)	105 (41.3%)	125 (49.2%)

Table 4
Selected laboratory data of co-infected malaria and HIV patients during the study period (n =254).

Lab data		Frequency (%)	Mean	Median	Range
Parasite density ($\times10^9$/L)	Low (0.1–0.6)	46 (18.1)	0.37±0.03	0.4	0.1–0.6
	Medium (0.7–1.2)	74 (29.1)	0.77±0.03	0.8	0.7–1.1
	High (> 1.25)	134 (52.8)	3.78±0.37	1.3	1.2–18.9
Haemoglobin (g/dL)	≤10	84 (33.1)	9.15±0.10	9.2	6.6–10.0
	>10	170 (66.9)	12.30±0.10	12.1	10.1–19.6
Platelet (1.25×10^9/L)	≤150	55 (21.7)	107.50±4.40	116.0	33.0–15030
	>150	199 (78.3)	250.00±5.10	229.0	151.0–517.0

count of 200 cells/μL and below. This is not surprising as the $CD4^+$ T cells are targeted for destruction by HIV virus in addition to the malaria parasite. Former reports mention that HIV-1 patients with a CD4 count 200 cells/μL have a higher risk of parasitemia or clinical malaria compared to those with a higher CD4 count[8,20]. Cellular mechanisms, including $CD4^+$ cells, responsible for protection against malaria, built up over a period of time as a result of repeated malaria attacks are destroyed in HIV infected individuals, thus leading to increased parasitemia and clinical malaria.

Our study revealed that majority (74.3%) of HIV subjects had malaria infection as opposed to only 25.7% who had no malaria infection and this was statistically significant. Being in a stable transmission area, the HIV positive individuals are more likely to be exposed to malaria hence, the increase prevalence of parasitemia cases seen in the study regardless of whether symptoms were present or not. This collaborates with other studies where there was an increase incidence, prevalence and severity of malaria in HIV infected individuals[18,21,22]. This is because HIV infection is known to lead to a progressive cellular immunosuppression with a resultant impairment in immune response to malaria.

Recent investigations suggest that HIV positive individuals with reduced CD4 cell count may be more likely at risk to present with malarial parasitemia[23]. In our study, majority of the subjects with low $CD4^+$ cell count of 200 cells/μL was significantly associated with high parasitemia. A similar study showed that individuals with $CD4^+$cell counts less than 200 cells/μL had six times the odds of having malaria compared with those with $CD4^+$ cell counts of at least 500 cells/μL[8]. The odds of having clinical malaria at a routine visit were reported to increase with advancing clinical stage of HIV-1 infection. This increase was most evident for visits of individuals in WHO stage 4, at which the odds were nine times those in stage 1.

Based on WHO clinical stages, there were an increasing number of patients with malaria infection with advancing HIV disease, although the setback here was the difficulty in separating stages 1 and 2. Majority of the co-infected patients (50.8%) had significant high parasitemia with 60 patients having hyperparasitemic counts ($>2.5\times10^9$/L parasite count); though we did not record any form of death during the study period. This may be because those with a high degree of acquired immunity may be able to sustain sufficient antitoxic immunity to avoid severe disease despite HIV-associated immunosuppression[24]. Hospital-based studies in Zambia and Burundi found increased malaria case fatality ratios in patients with HIV compared with HIV-seronegative individuals, but the small sample sizes did not allow firm conclusions to be drawn[25,26]. A study in rural Kwazulu-Natal, an area of unstable malaria reported that HIV-infected children were more likely to experience severe disease, coma and death[27]. However, the high parasite densities found in our study is in contrast to the low parasite densities reported in an urban cohort population in Uganda[28]. Investigators have speculated that because HIV infection increases parasitaemia and reduces the response to therapy, it will increase the reservoir of infection in the human population and hence increase transmission[21,29]. In all, they did not take into account the wide variation in immunosuppression found at different stages of HIV-1 infection.

Haematological abnormalities are considered a hallmark of malaria and reported to be most pronounced in plasmodium falciparum infection[30]. Anaemia is said to be a common feature of malaria and HIV independently. This study however, could not establish this assertion as majority (66.9%) of the coinfected patients had haemoglobin level > 10 g/dL as against 52.8% reported among HIV-positive population and characteristics of anaemia of chronic disease in a Nigerian study[31,32]. The mean value of (9.15±0.11) g/dL recorded in our study is similar to 9.17±1.67 recorded for parasitized HIV infected in another part of the country[33]. The pathogenesis of anaemia in plasmodial parasitized patients is complex and multifactorial. They are thought to result from haemolysis of parasitized red cells, exacerbated removal of parasitized red cells, depressed and ineffective erythropoiesis[34]. Similarly, thrombocytopenia has been reported as a classical feature of malaria and low platelet count is usually seen in 72% of patients with uncomplicated malaria[35]. The platelet count of most individuals in this study was within normal range. Again, this could be because all the co infected individuals were already on the HAART therapy. *In-vitro* studies have shown that some components of the human immune response to *Plasmodium falciparum* are modified by HIV-1 but that others are unaffected[10,36].

In conclusion, our findings indicate that there is an increased prevalence of malaria parasitemia among HIV infected individuals in an endemic region. With the HAART therapy already instituted in Nigeria, the next step presumably is effective prevention and interventions in form of regular malaria prophylaxis as this is known to reduce the risk of malaria in HIV infected patients especially in sub Saharan areas where coinfection is common.

Conflict of interest statement

We declare that we have no conflict of interest.

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